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ABSTRACT

Background: Smoke from the burning of biomass fuels has been linked with adverse pregnancy outcomes such as low birth weight, stillbirth, and prematurity.

Objective: To identify potential underlying mechanisms of adverse perinatal outcomes, we explored the association of placental pathology with household air pollution in pregnant women from urban/periurban Tanzania who cook predominantly with charcoal.

Methods: Between 2011 and 2013, we measured personal exposures to fine particulate matter ($PM_{2.5}$) and carbon monoxide (CO) over 72 hours among a cohort of Tanzanian pregnant women. Placentas were collected after delivery for examination. Placental pathologies of inflammatory, hypoxic, ischemic/hypertensive, infectious and thrombotic etiologies were diagnosed, blinded to exposure levels. Using multiple logistic regression, we explored the association of $PM_{2.5}$ and CO exposure with placental pathology.

Results: 116 women had personal air exposure measurements and placental histopathology available for analysis. $PM_{2.5}$ and CO exposures were moderate [geometric means (GSD) 40.5 $\mu g/m^3$(17.3) and 2.21 ppm (1.47) respectively]; 88.6% of $PM_{2.5}$ measurements exceeded WHO air quality guidelines. We observed an increase in the odds (per one unit increase in exposure on the ln-scale) of fetal thrombotic vasculopathy both with increasing $PM_{2.5}$ (aOR 5.5; 95% CI 1.1-26.8) and CO measurements (aOR 2.5; 95% CI 1.0-6.4) in adjusted models only. Fetal thrombotic vasculopathy also was more common among pregnancies complicated by stillbirth or low birth weight.

Conclusions: Fetal thrombosis may contribute to the adverse outcomes associated with household air pollution from cook stoves during pregnancy. Larger studies are necessary for confirmation.

INTRODUCTION

An estimated forty percent of the world's population relies on solid biomass fuels (wood, charcoal, crop residues) for cooking or heating.(Bonjour et al. 2013) The smoke generated from the combustion of these fuels is a complex mixture of gases and fine particles including many known harmful pollutants such as carbon monoxide (CO), suspended fine particulate matter ($PM_{2.5}$), nitrogen oxide and polycyclic aromatic hydrocarbons. Household air pollution from biomass burning is now acknowledged as a major contributor to the global disease burden.(Lim et al. 2012)

During pregnancy, exposure to biomass cooking smoke has been linked with a reduction in birth weight, an increase in stillbirth, and a rise in preterm births in a number of epidemiologic investigations.(Amegah et al. 2014) Despite a growing evidence base of reproductive harm from household air pollution, methodological shortcomings remain including challenges in exposure assessment and the potential for uncontrolled confounding. Pathological examination of placentas from pregnant women exposed to biomass cook smoke may identify lesions that are known to be associated with adverse pregnancy outcomes. Furthermore, specific lesions may shed light on the underlying pathophysiology and suggest targets for amelioration of risk. For these reasons, we aimed to characterize placental pathology associated with household air pollution among women cooking with biomass fuels in an African population.

We hypothesized that the placenta would demonstrate *inflammatory* lesions in the setting of high household air pollutant exposure, and specifically when levels of fine particulate matter exposure are high. Cardiopulmonary researchers have demonstrated both that inhaled ultrafine particles can enter the circulation and that systemic measures of inflammation increase following exposure to air pollution.(Scapellato and Lotti 2007; Donaldson et al. 2005;Sørensen et al. 2003)

We posited that damage to the placenta may occur directly from exposure to circulating particles or secondary to systemic inflammation, either of which may result in any of the well-defined histologic placental chronic inflammatory lesions-- chronic villitis (Figure 1), chronic chorioamnionitis or intervillositis. Secondly, we hypothesized that biomass smoke exposed placentas would demonstrate *hypoxic* lesions, particularly as CO levels increase. CO impairs placental oxygen transport by increasing levels of carboxyhemoglobin thereby displacing oxygen from hemoglobin and reducing its availability to the fetus.(Soothill et al. 1996) The placental response in the setting of hypoxia is an increase in the surface area and vascularity of the villi. (Altshuler 1984) This adaptive angiogenesis is manifest as chorangiosis (Figure 2), a common feature of tobacco-exposed placentas.(Pfarrer et al. 1999)

METHODS

Overview

Between January 2011 and May 2013, personal exposures to fine particulate matter ($PM_{2.5}$) and carbon monoxide (CO) were measured during the second or third trimester for a subset of pregnant women enrolled in the The Prenatal Iron Supplements study (NCT01119612; http://clinicaltrials.gov/show/NCT01119612). This parent trial recruited only HIV negative women in their first or second pregnancies living in an urban/periurban setting in and around Dar es Salaam, Tanzania.(Etheredge, AJ et al. 2015) For our substudy on household air pollution, we approached only nonsmoking pregnant women who were the primary cooks in their household and 15 years of age or older to participate in exposure measurement. Study subjects answered a number of questions specific to their cooking practices including types of fuel used, hours spent cooking, stove design, cooking area ventilation, smoking habits of other household members, as

well as exposure to other sources of pollution such as traffic, tobacco, incense, and the burning of rubbish. As part of the parent trial, standard operating procedures for gestational age assessment, birth measurements, and collection of the placenta were followed by trained research staff. Placental malaria was the primary outcome of the The Prenatal Iron Supplements study and thus trial procedures were optimized to encourage delivery at one of the study affiliated facilities to enable collection of the placenta. Furthermore, the placentas were processed for histopathologic examination regardless of whether pregnancy complications occurred and therefore are more representative of the general population in this study.

Subjects were eligible for the analysis presented in this manuscript if a personal exposure measure of either $PM_{2.5}$ or CO was successfully obtained during pregnancy and placental histopathology was available. The study protocol was approved by the Institutional Review Boards of Muhimbili University of Health and Allied Sciences, the Harvard T. H. Chan School of Public Health, and Partners Healthcare (for Massachusetts General Hospital). Informed consent was obtained from all participants.

Exposure monitoring

CO was measured over 72 hours using passive diffusion tubes (Draeger Carbon Monoxide 50/a-D, Draeger USA) clipped to the woman's clothing and measurements of the length of color change were used to calculate an average concentration in parts per million (ppm). $PM_{2.5}$ exposure was measured during the first and third 24 hours of the 72-hour sampling period using a portable pump worn by the subject. Filters were weighed on a Mettler Toledo MT5 microbalance after conditioning in a temperature and humidity controlled environment for at least 24 hours and

statically discharged via a polonium source. All filter weights took into account correction for lab blanks. The two 24-hr $PM_{2.5}$ mass concentrations were averaged to represent the personal fine particulate matter exposure. Exposure measurements were conducted only once per subject during a single 72 hour period in either the second (12%) or third (88%) trimester of pregnancy. For additional details regarding CO and $PM_{2.5}$ measurements, see Supplemental Material ("Details of carbon monoxide exposure measurement" and "Details of fine particulate matter exposure measurement").

Placental sampling, histopathologic processing and interpretation

Prior to commencement of the study, research nurses received hands-on training and skills verification in placental sampling by a US perinatal pathologist (DJR). Similarly, study pathology technicians were trained and supervised by a US perinatal pathologist (DJR). Following birth, portions of the umbilical cord, membranes, and three full thickness sections of a pre-specified size (approximately 3 cm^3) from the placental disk were obtained by study nurses. Palpable or visible abnormalities of the placental disk were preferentially targeted for sampling. The cord and membranes were subsequently excised and the placenta weighed with an electronic scale to the nearest gram. All placental samples obtained were placed in 10% neutral buffered formalin and then transported to the pathology lab at Muhimbili University where they remained in fixation for at least 4 hours up to a maximum of 24 hours. Following fixation, samples were trimmed and routinely processed to produce hematoxylin/eosin stained slides. All births occurred in one of the study hospitals. Study nurses were stationed at study facilities around the clock to complete placental sampling in a timely manner. Sampling occurred within the first hour after delivery for

most subjects and if this could not be accomplished, placentas were placed in a facility refrigerator until sampling occurred.

Slides were shipped to Massachusetts General Hospital where they underwent histopathologic examination by an experienced perinatal pathologist (DJR) who was blinded to details regarding cooking fuel and measured exposure levels. Between one and three parenchymal slides were available for review. Diagnoses were rendered using standard diagnostic criteria (Roberts 2008; Roberts 2013; Chisholm and Heerema-McKenney 2015; Redline et al. 2004a; Redline et al. 2004b; Redline et al. 2003; Altshuler 1984; Ogino and Redline 2000; Schwartz 2001) and coded using a standardized form. Diagnoses were made on the routine hematoxylin and eosin stained slides; no special studies were obtained. Specific lesions were assigned into the categories of hypoxic, hypertensive/ischemic, inflammatory, infectious, and thrombotic as outlined in Table 1. Thrombotic lesions were further subclassified as fetal or maternal in origin. More than one diagnosis could be rendered for a given subject. Although our specific hypothesis was that the placenta will show adaptations related to inflammation or hypoxia, all other histopathologic findings were noted and compared with exposure data.

Placental weights were compared against a United States reference standard.(Pinar et al. 1996) as placental weight standards for Tanzania currently do not exist. While the relevance of this standard to Tanzanian placentas is unclear, extremes of the standard should remain relevant. Placentas were therefore characterized as small for gestational age ($<10^{th}$ percentile), large for gestational age ($>90^{th}$ percentile), or appropriately sized. Gestational age was assigned in the parent trial by means of a postnatal new Ballard examination conducted by facility-based research staff within 24 hours following delivery.(Ballard et al. 1991) The total of both the neurologic and

external features scores was translated into gestational age in weeks using the published Ballard maturity-rating tables.

Data Analysis

Subjects were grouped into tertiles of exposure for both $PM_{2.5}$ and CO to evaluate exposure-response associations. The Cochran Armitage trend test was used to evaluate whether the prevalence of placental lesions in a given category (hypoxic, ischemic/hypertensive, inflammatory, infectious, thrombotic-maternal, thrombotic-fetal) increased across exposure tertiles and two-sided p-values reported. If any cell count was less than 5, an exact p-value was reported rather than the asymptotic approximation. Similar analyses were performed to compare the proportion of small for gestational age placental weights and large for gestational age placental weights across tertiles of $PM_{2.5}$ and CO exposure.

Models were also fit using multiple logistic regression with exposure represented as a continuous predictor of placental lesion or placental weight categories. Exposure measurements for both $PM_{2.5}$ and CO were *ln*-transformed given the skews in measurement distributions. Candidate covariates were selected *a priori* and included maternal age (18-20, 21-25, $\geq$ 26 years), body mass index (<18.5, 18.5-24.9, 25-29/9. 30+ kg/m^2), second hand smoke exposure, season of exposure measurement (rainy vs dry), and a household asset index (low: 0-5, medium: 6-8, high: 9-10) constructed after tallying household ownership of ten items (car, generator, bicycle, sofa, television, radio, refrigerator, fan, electricity, and potable aqua). Additional covariates were considered including maternal hypertension, antenatal treatment for malaria and tobacco use during pregnancy but were not included in the adjusted models given the low prevalence of these

conditions and the consequent destabilization of effect estimates. No adjustments were made for multiple comparisons. Finally, we explored associations between each of the placental lesion categories and adverse pregnancy outcomes, specifically low birth weight (<2500 grams) and stillbirth, calculating unadjusted odds ratios and 95% confidence intervals were estimated using logistic regression for both stillbirth and low birth weight.

Analyses were performed separately for $PM_{2.5}$ and CO exposures. Statistical analyses were performed using SAS software version 9.4.

RESULTS

Of the 239 primigravid or secundigravid women recruited for $PM_{2.5}$ and CO exposure monitoring during pregnancy in the larger study, 116 had placental slides available for histopathologic review and comprise our cohort for the present analysis. All 116 had CO measurements successfully obtained. $PM_{2.5}$ measurements were available for 79 of the 116 (see Supplemental Material, Figure S1). Exposure measurement occurred during 2011 and 2012 for all subjects except one who was recruited during 2013. Maternal characteristics, sociodemographics, cooking behaviors, kitchen characteristics, and other sources of household air pollution are summarized in Table 2. The women were recruited primarily from urban or periurban households in and around Dar es Salaam, Tanzania. One hundred ten of 116 (94.8%) were the primary cooks in their household and the majority cooked three meals per day (74 of 116, 63.8%). Cooking areas were commonly shared with other families (54 of 116, 46.6%). Almost one third of the cooking areas were located outdoors (37 of 116, 31.9%); this varied somewhat depending on the rain. Approximately half of the outdoor cooking areas were located under a roof and the remainder in the open air. For those cooking inside, a separate cooking area located in a different structure from

the main house was utilized by about one third of the women (34%-37% depending on the season). Almost all indoor cooking spaces were ventilated by at least one window or an open door. The primary household fuel was charcoal in both the dry (93 of 116, 80.2%) and rainy seasons (62 of 116, 53.4%). Kerosene was the second most common fuel with use increasing during the rainy season (46 of 116, 39.6%) compared to the dry season (12 of 116, 10.3%). Only two subjects cooked with gas or electricity and fewer than 10 households used wood as the primary fuel. When this cohort of 116 subjects was compared with the 239 subjects that comprised the larger study on exposure to household air pollutants during pregnancy, there were no significant differences identified with regards to any of the characteristics presented in Table 2 (data not shown).

Exposure levels

Among the 79 subjects with $PM_{2.5}$ measurements, the geometric mean personal $PM_{2.5}$ exposure was 40.5 $\mu g/m^3$ (geometric standard deviation: 17.3) with a range from 14.9 to 528.2 $\mu g/m^3$. The first tertile of exposure included exposures up to 32.9 $\mu g/m^3$, the second tertile from 33 to 45.6 $\mu g/m^3$, and the upper tertile above 45.6 $\mu g/m^3$. The geometric mean personal CO exposure for the 116 women with these measurements was 2.21 ppm (geometric standard deviation: 1.47) and ranged from 0.34 ppm to 25.15 ppm. The first tertile included exposures up to 1.42 ppm, the second tertile from 1.43 to 3.06 ppm, and the upper tertile above 3.06 ppm. As with baseline characteristics, exposure measurements did not differ significantly between subjects with available placental slides compared with the larger group of 239 subjects (data not shown). The strength of correlation between $PM_{2.5}$ and CO measurements for an individual subject was only

weakly positive (Pearson's r=0.30, p=0.008 after *ln*-transformation) and therefore we continued with separate analyses for $PM_{2.5}$ and CO.

Association of exposure with placental pathology

Inflammatory lesions: We did not observe a statistically significant association between inflammatory lesions and $PM_{2.5}$ (Table 3) although there was an increased prevalence of inflammatory placental lesions across tertiles of $PM_{2.5}$ (1^{st} tertile, 11.1%; 2^{nd} tertile, 15.4%; 3^{rd} tertile, 26.9%; p=0.15 for trend).The prevalence of chronic villitis (Figure 1), independently from the remainder of the inflammatory lesions, also varied by $PM_{2.5}$ tertile but similarly did not reach statistical significance (1^{st} tertile, 7.4%; 2^{nd} tertile, 11.5%; 3^{rd} tertile, 19.2%; p=0.22 for trend). No association was discernible for CO exposure and inflammatory placental lesions (p=0.42 for trend) (Table 4). Similarly, no association was observed between inflammatory placental lesions and either $PM_{2.5}$ or CO when exposure was considered a continuous predictor after *ln*-transformation in adjusted logistic models (Tables 3 and 4). Of note, our cohort was recruited from a placental malaria study and placental malaria is associated with inflammatory placental changes, specifically chronic villitis. However, placental malaria was rare in our cohort (4 of 116, 3.4%). No active malaria was detected, only the presence of malaria pigments indicative of chronic malaria. Furthermore, none of these four cases demonstrated chronic villitis, intervillositis or other inflammatory lesions.

Hypoxic lesions: Hypoxic placental lesions were common in the overall cohort (25 of 116, 21.5%); however, the frequency of hypoxic lesions did not vary by either $PM_{2.5}$ or CO exposure (p=0.41 and 0.53 respectively) (Tables 3 and 4). Analysis of chorangiosis or edema separately,

rather than together under the broader category of hypoxic lesions, did not alter our findings. Similarly, no association was observed between hypoxic placental lesions and either $PM_{2.5}$ or CO when exposure was considered a continuous predictor after *ln*- transformation in adjusted logistic models (Tables 3 and 4).

Other placental pathology: We identified associations between fetal thrombotic vasculopathy (FTV) (Figure 3) and both $PM_{2.5}$ and CO. The adjusted odds of FTV was 5.5 times higher (95% CI 1.1, 26.8) per one unit increase in the *ln*-transformed $PM_{2.5}$ measurements after adjustment; notably, the unadjusted effect size was lower and did not reach statistical significance (OR 2.8, 95% CI 0.9, 8.9) (see also Supplemental Material, Table S1). While the trend test across tertiles of $PM_{2.5}$ did not reach statistical significance (p=0.22), the prevalence of FTV was much lower in the first tertile of exposure (3.7%) compared with the upper two tertiles (19.2% and 15.4%) (Table 3). For CO, we observed a trend across tertiles of exposure (1^{st} tertile, 5.1%; 2nd tertile 7.7%; 3^{rd} tertile, 21.1%; p=0.03)(Table 4) and an increase in the odds of diagnosing FTV (aOR 2.5, 95% CI 1.0, 6.4) with increasing *ln*-transformed CO exposure after covariate adjustment although the latter did not quite reach statistical significance in adjusted or unadjusted models (Supplemental Material, Table S2). A pattern in the severity or location (large vessel versus distal vessel) of the fetal thrombotic lesions by tertile of $PM_{2.5}$ or CO exposure was not discernible.

We also observed an inverse association between $PM_{2.5}$ and infectious placental lesions when considering $PM_{2.5}$ as a continuous predictor (Table 3). This was driven by a reduction in the prevalence of chorioamnionitis across tertiles as we observed chronic malaria (pigment) in only four subjects, two of whom had no available $PM_{2.5}$ measurements and the other two with measurements in the uppermost tertile. No other trends were observed for either $PM_{2.5}$ or CO

exposure including no identified associations with small or large for gestational age placental weights. In sensitivity analysis, neither fuel type (kerosene versus charcoal) nor season was associated with any of the considered placental lesion categories (data not shown).

Association of placental pathology with adverse birth outcomes

We explored associations between placental lesion categories and both low birth weight (<2500 grams) and stillbirth. Preterm birth (<37 weeks at delivery) was not considered as only 2 of the 116 in our cohort were identified as delivering a preterm infant. Information on preeclampsia was not routinely available from facility records and study nurses did not obtain blood pressure measurements at delivery. Stillbirth was diagnosed in 5 pregnancies (4.3%) and low birth weight in 13 pregnancies (11.2%) A placenta with fetal thrombotic vasculopathy was associated with an increased odds of both low birth weight (unadjusted OR 4.59; 95% CI 1.2, 17.9) and stillbirth (unadjusted OR 6.7; 95% 1.0, 44.7). None of the other placental lesion categories were associated with either low birth weight or stillbirth with the exception of placentas demonstrating hypertensive/ischemic lesions which were associated with stillbirth.

DISCUSSION

With this investigation, we used placental pathology to further evaluate the observed epidemiologic associations of household air pollution with adverse pregnancy outcomes and explore potential mechanisms underlying these effects. We did not identify a statistically

significant association between $PM_{2.5}$ and placental inflammation or between CO exposure and placental hypoxia as hypothesized. Instead, we observed that thrombotic placental lesions were linked with both $PM_{2.5}$ and CO exposures in our cohort. The association of household air pollutant exposure with thrombotic lesions we detected was limited to the fetal circulation of the placenta, manifest as fetal thrombotic vasculopathy, whereas maternal thrombotic lesions did not vary by exposure to either pollutant. It is possible that the fetus and/or placenta may be particularly vulnerable to household air pollution exposures. While the prevalence of placental pathologic findings in the general population are for the most part not quantified as most placentas do not receive a pathologic examination in the absence of pregnancy complications, one study reported the incidence of fetal thrombotic lesions to be 3% in 169 consecutively examined placentas.(Vern 2000) In comparison, the frequency with which we observed fetal thrombotic vasculopathy in the uppermost tertile of both $PM_{2.5}$ and CO exposure was remarkably high (15.4% and 21.1% respectively).

We also observed an increased risk for low birth weight and stillbirth in pregnancies complicated by placental fetal thrombotic vasculopathy. Prior literature has similarly linked fetal thrombotic vasculopathy with adverse perinatal outcomes including oligohydramnios, growth restriction, stillbirth as well as coagulopathy and systemic thrombosis in the newborn.(Saleemuddin et al. 2010; Chisholm and Heerema-McKenney 2015) Additionally, severe fetal thrombotic vasculopathy has been connected with intracranial hemorrhage and neurologic impairment of the infant.(Chisholm and Heerema-McKenney 2015) To date, the impact of prenatal exposure to household air pollution on the neurocognitive development of the infant has been understudied although preliminary work suggests adverse effects.(Dix-Cooper 2012) We propose this should be an area of intensified study. An additional benefit to be

achieved through improvements in household air pollution may be reducing harms to the developing fetal brain.

The strength of this study lies in access to both personal exposure measurements during pregnancy and placental pathology. Measured exposures to fine particulate matter in our urban/periurban population using predominantly charcoal and kerosene for cooking were lower compared to exposures reported from rural populations using mostly wood fuel.(Dionisio 2012; Van Vliet 2013; Smith 2010; Jiang and Bell 2008) Exposure to carbon monoxide was also lower for our subjects compared to rural biomass users as with fine particulate matter but the differences were less pronounced than for fine particulate matter (geometric mean of 2.21 ppm in our study versus 2.38 ppm for women cooking on open fires in rural Guatemala).(Naeher et al. 2001) That said, the exposures we observed were still moderate to high and the majority of the women (70/79, 88.6%) exceeded the World Health Organization guideline for acceptable air quality (mean 24-hour $PM_{2.5}$ not to exceed 25 $\mu g/m^3$).(World Health Organization 2010) The lowest exposure tertiles are therefore not 'unexposed.' In addition, we measured exposure only once during the latter half of pregnancy which may not fully characterize gestational exposure. Exposure earlier in pregnancy might be more closely related to placental injury as this is when trophoblasts transform uterine spiral arteries and establish uteroplacental blood flow.(Pijnenborg et al. 1980)

An additional limitation is that we measured exposure to fine particles with a diameter smaller than 2.5 micrometers. It may be that ultrafine particles, with diameters smaller than 100 nanometers, are the more relevant exposure for the placenta; our study was not designed to assess this. For inflammation in the placenta to be linked with inhaled exposure to particulate matter, particles must migrate across alveolar spaces into the systemic circulation leading to

direct toxicity of distal tissues (e.g., placenta) or localized inflammation in lung tissues leads to systemic inflammation. Similar hypotheses have been advanced to explain the pathophysiology underlying the relationship between ambient air pollution, particulate matter, and cardiovascular health.(Brunekreef and Holgate 2002; Donaldson et al. 2005) Experiments exposing rats via inhalation to radiolabeled ultrafine particles have demonstrated that particles can be found outside of pulmonary tissues such as in the liver and spleen although excretion by the kidneys was notably rapid.(Kreyling et al. 2002) Particle composition may also be critical to toxicity (Lippmann et al. 2013) and we did not specifically characterize the chemical composition of the fine particles we measured. These limitations may have contributed to the lack of formal statistical significance between exposure and inflammatory or hypoxic placental lesions as initially hypothesized.

A link between personal measures of exposure to household air pollution and fetal placental thrombosis, although not originally hypothesized, is plausible given interconnections between inflammatory reactions and the clotting cascade. Coagulation, inhibition of fibrinolysis, and platelet aggregation has been demonstrated following exposure to particulate matter in both in vitro laboratory investigations and animal models.(Nemmar et al. 2002; Gilmour et al. 2005) We formally tested a number of hypotheses evaluating associations of both $PM_{2.5}$ and CO with each of the various placental lesion categories and did not adjust for multiple comparisons. That we identified the same lesion, fetal thrombotic vasculopathy, for both $PM_{2.5}$ and CO and in both categorical and continuous analyses does strengthen our findings. That said, the exploratory nature of our work, the wide confidence intervals in effect sizes, and the lack of consistency between categorical and continuous outcomes or between adjusted and unadjusted analyses underscores the need for further investigation in larger studies.

CONCLUSIONS

In summary, our results suggest that prenatal exposure to inhaled particulate matter and carbon monoxide may be associated with fetal thrombosis in a dose-dependent matter. Our findings should be verified in larger studies with populations using a variety of biomass fuels in both rural and urban settings. Attention should be paid to obtain repeated measures of exposure over the course of gestation. Fetal thrombotic vasculopathy may contribute to unfavorable perinatal outcomes like low birth weight and stillbirth, outcomes that have been associated with household air pollution exposure in pregnancy. Moreover, given a recognized link between fetal thrombosis and adverse neurocognitive development, our findings suggest additional long-term benefits should be evaluated following reductions in prenatal household air pollution exposure.

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Table 1 Categorization of Placental Lesions

Hypoxic		Chorangiosis Edema
Ischemic/ Hypertensive*		Infarcts Distal villous hypoplasia Decidual vasculopathy Villous agglutination Abruption
Inflammation (w/o infection)		Chronic villitis Intervillositis Chronic chorioamnionitis
Infection		Acute Chorioamnionitis (maternal and/or fetal) Malaria
Thrombotic	Maternal	Increased perivillous fibrin Massive perivillous fibrin distribution Maternal floor infarct Intervillous thrombi Subchorionic thrombus
	Fetal	Fetal thrombotic vasculopathy
Other		Meconium Amniotic metaplasia (+/- clear cell) Calcifications Maternal sickling Villous dysmaturity

* Given the overlap of ischemic and hypertensive lesions, these were grouped together for analysis.

Table 2 Maternal characterics, residential environment, cooking behaviors, and other sources of household air pollution

	Overall cohort n=116
Maternal characteristics	
Age category (years)	
18-20	36 (31.0)
21-25	48 (41.4)
≥ 26	32 (27.6)
Parity	
Primigravid	73 (62.9)
Secundigravid	43 (37.1)
BMI category (kg/m^2)	
< 18.5	7 (6.0)
18.5-24.9	70 (60.3)
25-29.9	27 (23.2)
≥ 30	12 (10.3)
History of hypertension	5 (4.3)
Treated for antepartum malaria episode	0 (0)
Sociodemographics	
Housing	
Apartment/ multifamily compound	93 (80.5)
Single family home	22 (19.5)
Neighborhood	
Urban	41 (35.3)
Periurban or rural	75 (64.7)
Household asset index	
Low	6 (5.2)
Medium	62 (53.5)
High	48 (41.4)
Cooking behaviors during measurement	
Cooked meals for family	111 (96.5)
Fuels used	
Did not cook	4 (3.5)
Wood only	2 (1.7)
Charcoal only	39 (33.9)
Kerosene only	13 (11.3)
Both charcoal and kerosene	55 (47.8)
Gas or electricity	0 (0)
Other mixtures	2 (1.7)
Rainy season during measurement	59 (50.9)
Cooks for commerce	2 (1.7)
Kitchen characteristics	
No. of stoves	2.0 (0.0) [a]
Cooking area shared with other	54 (46.6)

families	
Cooking area outdoors/partially outdoors	37 (31.9)
Visible soot on walls	95 (83.3)
Other sources of household air pollution	
Use of incense	23 (19.8)
Use of mosquito coils	8 (6.9)
Burning of rubbish	20 (17.4)
Secondhand smoke	17 (14.7)
Tobacco use	3 (2.6)
Nearest road is paved	34 (30.9)

Values are n (%) unless otherwise noted. Numbers may not add to sample size because of missing values.

[a] Median (interquartile range).

Table 3 Placental pathology by particulate matter exposure

	$PM_{2.5}$ exposure				
	1st Tertile n=27	2nd Tertile n=26	3rd Tertile n=26	Significance [a]	Adjusted OR[b] (95% CI)
Placental lesion categories					
Hypoxic	7 (25.9%)	6 (23.1%)	8 (30.8%)	0.69	1.4 (0.5, 4.0)
Ischemic/ Hypertensive	1 (3.7%)	4 (15.4%)	2 (7.7%)	0.64	0.7 (0.1, 6.0)
Inflammatory	3 (11.1%)	4 (15.4%)	7 (26.9%)	0.15	1.8 (0.6, 5.6)
Infectious	7 (25.9%)	4 (15.4%)	3 (11.5%)	0.21	0.1 (0.0, 0.7)
Thrombotic (maternal)	3 (11.1%)	7 (26.9%)	3 (11.5%)	1.00	2.5 (0.7, 8.6)
Thrombotic (fetal)	1 (3.7%)	5 (19.2%)	4 (15.4%)	0.22	5.5 (1.1, 26.8)
Placental Weight					
Small for gestational age	9 (39.1%)	10 (50.0%)	10 (55.6%)	0.29	1.5 (0.5, 4.8)
Large for gestational age	2 (8.7%)	0 (0.0%)	2 (11.1%)	1.00	2.01 (0.3, 12.7)

CI= confidence interval. OR= odds ratio. $PM_{2.5}$ = fine particulate matter < 2.5 micrometers.

[a] Cochran Armitage Trend test, two sided p-values. Exact p-values reported when cell counts < 5.

[b]The odds ratios represent the odds of having a placental lesion in the considered category (e.g., hypoxic) for a one unit increase in $PM_{2.5}$ exposure on the *ln*-scale adjusted for age, body mass index, second hand smoke exposure, season of exposure measurement (rainy vs dry), and a household asset index.

Table 4 Placental pathology by carbon monoxide exposure

	CO exposure				
	1st Tertile n=39	2nd Tertile n=39	3rd Tertile n=38	Significance [a]	Adjusted OR [b] (95% CI)
Placental lesion categories					
Hypoxic	8 (20.5%)	9 (23.2%)	8 (21.1%)	0.95	1.0 (0.5, 1.9)
Ischemic/ Hypertensive	5 (12.8%)	3 (7.7%)	4 (10.5%)	0.73	1.0 (0.4, 2.6)
Inflammatory	5 (12.8%)	10 (25.6%)	6 (15.8%)	0.73	1.4 (0.6, 3.0)
Infectious	9 (23.1%)	9 (23.1%)	7(18.4%)	0.62	0.7 (0.3, 1.6)
Thrombotic (maternal)	8 (20.5%)	5 (12.8%)	6 (15.8%)	0.57	0.8 (0.4, 1.9)
Thrombotic (fetal)	2 (5.1%)	3 (7.7%)	8 (21.1%)	0.03	2.5 (1.0, 6.4)
Placental weight					
Small for gestational age	10 (33.3%)	10 (30.3%)	15 (46.9%)	0.26	1.2 (0.6, 2.4)
Large for gestational age	5 (16.7%)	5 (15.2%)	3 (9.4%)	0.47	0.7 (0.2, 2.0)

CI= confidence interval. CO= carbon monoxide. OR= odds ratio.

[a]Cochran Armitage Trend test, two sided p-values. Exact p-values reported when if a cell count < 5.

[b]The odds ratios represent the odds of having a placental lesion in the considered category (e.g., hypoxic) for a one unit increase in CO exposure on the *ln*-scale adjusted for age, body mass index, second hand smoke exposure, season of exposure measurement (rainy vs dry), and a household asset index.

FIGURE LEGENDS

Figure 1: Representative sample of chronic villitis (long arrow) and normal non-inflamed villous (arrowhead). Note increased stromal cellularity and decreased vascularity in villi affected by chronic villitis. 20X

Figure 2: Representative sample of chorangiosis. Mature chorionic villi showing increased numbers of capillaries meeting diagnostic criteria for chorangiosis. 40X

Figure 3: Representative samples of fetal thrombotic vasculopathy. A (top image). 20X hematoxylin and eosin stained section of placenta showing normally vascularized villi (arrow) and adjacent field of avascular villi, distal vessel-fetal thrombotic vasculopathy (arrowheads). B (bottom image). 40X hematoxylin and eosin stained section of a stem villous vessel showing an endothelial cushion with a cap of fibrin clot, large vessel-fetal thrombotic vasculopathy, arrow.

Figure 1.

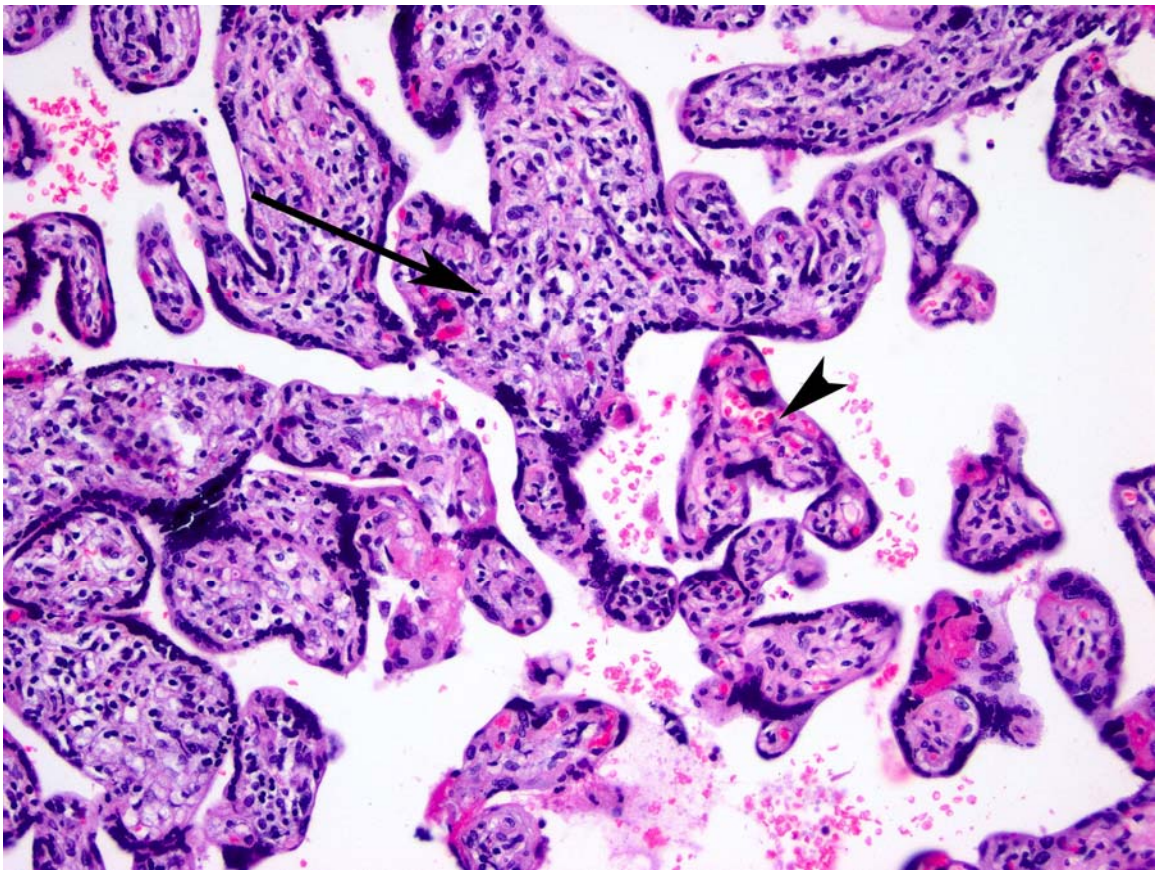

Figure 2.

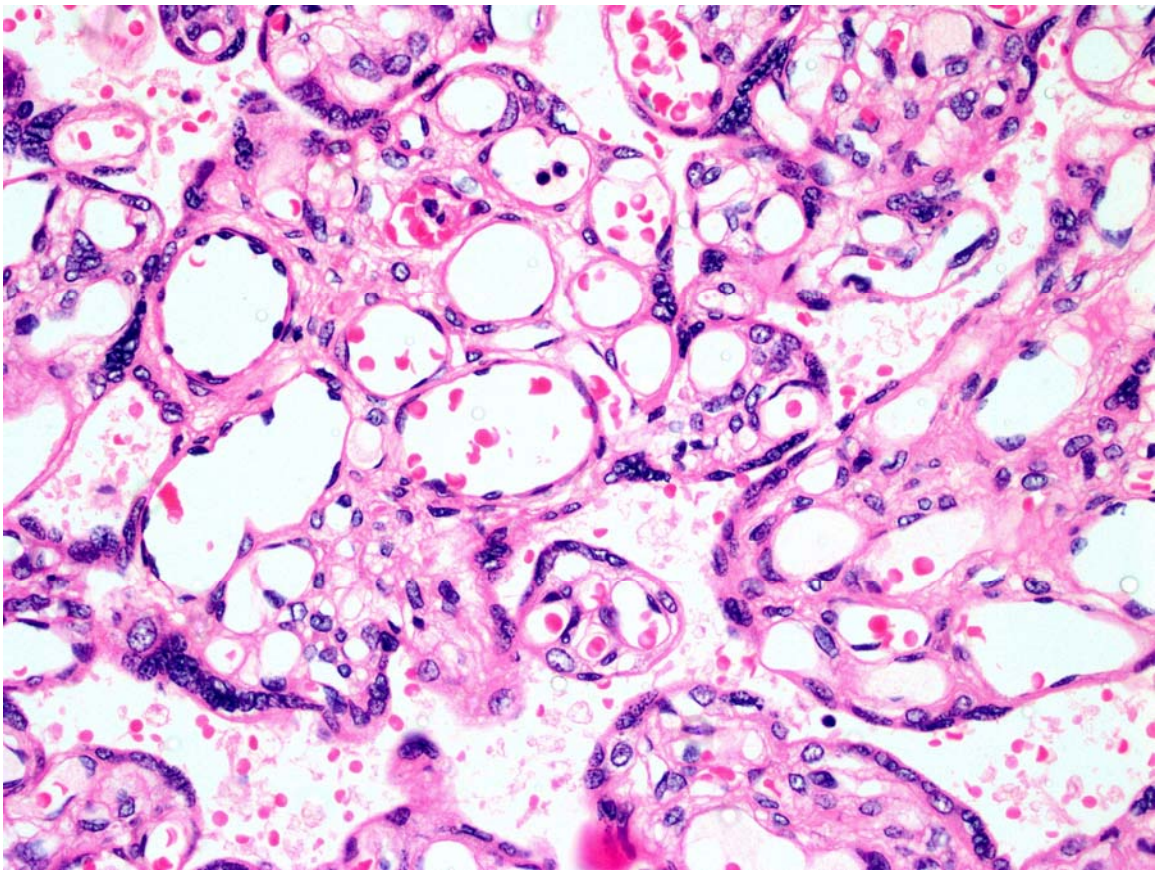

Figure 3.